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The Analysis of Aqueous Mixtures using Liquid Chromatography - Electrospray Mass Spectrometry

by

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To my parents, for their constant support.

TABLE OF CONTENTS

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ABSTRACT

The focus of this dissertation is the use of chromatographic methods coupled with electrospray mass spectrometry (ES-MS) for the determination of both organic and inorganic compounds in aqueous solutions.

The combination of liquid chromatography (LC) methods and ES-MS offers one of the foremost methods for determining compounds in complex aqueous solutions. In this work, LC-ES-MS methods are devised using ion exclusion chromatography, reversed phase chromatography, and ion exchange chromatography, as well as capillary electrophoresis (CE). For an aqueous sample, these LC-ES-MS and CE-ES-MS techniques require no sample preparation or analyte derivitization, which makes it possible to observe a wide variety of analytes as they exist in solution. The majority of this work focuses on the use of LC-ES-MS for the determination of unknown products and intermediates formed during electrochemical incineration (ECI), an experimental waste remediation process.

Ion exclusion chromatography (IEC) was used to study small carboxylic acids. The effect of pH, solvent composition, sheath gas make-up, and presence of concentrated matrix was observed. Detection limits were found to range from 40 to 200 ppb for the direct infusion of most carboxylic acids and from 2 to 8 ppm for IEC-ES-MS.

The ECI of benzoquinone is studied using IEC-ES-MS. Unknown intermediates and products are identified and observed as a function of ECI time. The ECI procedure is described in detail and electrode efficiency is evaluated. Mechanisms are also proposed for the production of maleic, succinic, malonic, and acetic acids.

The ECI of 4-chlorophenol is observed using reversed phase liquid chromatography - ES-MS to study aromatic compounds, and ion exchange chromatography - ES-MS for organic and inorganic ions. The majority of this work is accomplished with ion exchange chromatography - ES-MS, which requires a suppressor to remove sodium ions from the mobile phase. The system has proven effective in the identification of a wide range of unknown compounds in complex aqueous solutions. Detection limits range from 50 ppb to 4 ppm.

Capillary electrophoresis was also studied as a possible separation method coupled to an ES-MS. Different mobile phases are examined and tested with CE-ES-MS for the determination of carboxylic acids. Detection limits range between 1 and 10 ppm.

CHAPTER 1. GENERAL INTRODUCTION

There is tremendous potential in coupling the resolving capability of liquid chromatography (LC) with the structural information and specific detection of electrospray - mass spectrometry (ES-MS). The resulting combination, LC-ES-MS, is a powerful analytical technique capable of accurate identification of unknown compounds in mixtures. LC used with traditional absorption detection methods does not yield the selectivity of LC-ES-MS. The widely used gas chromatography - mass spectrometry (GC-MS) methods are inadequate for nonvolatile, thermally unstable, and polar compounds [1]. GC-MS often requires preparation and derivatization of the sample before it can be injected onto the column. LC-ES-MS requires no preparation or derivatization, which makes it possible to observe a wide variety of analytes as they exist in aqueous solutions.

Historical Perspective of LC-MS and Interface Developments

The combination of LC and MS offers the analytical chemist one of the most powerful techniques for the determination of unknown compounds. Attempts to couple LC and MS systems on-line by various laboratories began in the 1960's [2], but were unsuccessful due to technological problems that could not be overcome at the time. Some researchers considered off-line techniques with independent collection of liquid fractions of effluent, evaporation of solvent, and transfer of solute to MS as the only realistic method of using LC and MS together [3-5]. Successful on-line LC-MS results were not reported until the 1970's by the laboratories of E. C. Horning, F. W. McLafferty, and R. P. W. Scott [6-8]. The first successful commercially available LC-MS interface was the transport, or moving-belt, system designed by McFadden [2,9] in which the chromatography effluent is deposited on a moving stainless steel belt which transports the sample to the mass spectrometer. The effluent solvent in the transport system is removed in a vacuum before the analyte reaches the ion source and is volatilized. The moving belt system was utilized and improved for over a decade [4,5,10-13].

Direct liquid injection (DLI) is one method of on-line LC-MS [14-18]. It was first developed in the laboratory of F.W. McLafferty [14] with similar work reported later by Henion [18]. In a DLI method, the highest fraction of HPLC effluent compatible with the vacuum system of the MS is introduced into the MS ion source. The gas flow produced from the effluent of the conventional LC column was twenty times higher than typical vacuum system could withstand at the time of the early DLI experiments, so significant quantities of the effluent were split from the flow, and only a small fraction, 1-5%, went to the MS. The requirement of such a large split seriously reduced the usefulness of DLI-LC-MS. Cryogenic pumps with improved vacuum efficiency were utilized to handle higher flow rates of up to 100 μl/min which increased the use of DLI methods somewhat [16,17,19,20].

Many developments over the past twenty years have improved the performance of LC-MS. Some of the greatest improvements have resulted from advancements in micro HPLC techniques [21-26] which reduce the flow from the column to 10 - 50 μl/min and eliminate the need for splitting. Another area of significant advancements in LC-MS has been the development of effective new systems to interface LC and MS. The fundamental problem of LC-MS with conventional flow rates has been the inability of the mass spectrometer vacuum system to withstand all the solvent vapor of the evaporated effluent. Thermospray and atmospheric pressure ionization (API) sources have been developed to successfully eliminate vacuum load difficulties in LC-MS.

Thermospray

The thermospray ionization interface [27-29] significantly increased the acceptance of LC-MS, because it readily accommodates conventional reversed phase flow rates and eluent compositions, and it is a relatively simple and rugged system that does not require unusual training to operate. The technique was first developed by Vestal and co-workers [27] in 1983. In thermospray ionization an aqueous sample is passed through a heated metal capillary tube. Ions are produced by direct ion evaporation [30] of a sample ion or by a two-step process similar to conventional chemical ionization (CI), where an ion of electrolyte, typically ammonium acetate, ejected from a droplet reacts with a sample molecule in the gas phase to generate a sample ion. Thermospray uses an additional vacuum line directly at the ion source to maintain vacuum while accommodating up to 2 ml/min of effluent flow rate. The technique produces accurate molecular weight information but thermospray's limitations include difficulty in sensitive temperature control, thermal degradation, poor detection limits, and inability to handle low liquid flow.

API sources

API sources create droplets and ions from LC effluent in an atmospheric pressure region and then draw ions into a MS. The first work using API sources was done by Horning and his co-workers [31-35]. Horning used heated nitrogen gas to volatize the sample and thermal electrons of a corona discharge to ionize the sample. The development of API sources has grown rapidly over the past 15 years and three API sources will be discussed here: heated pneumatic nebulizer, liquid ion evaporation, both originally reported in 1983, and electrospray, first developed in 1984.

Heated pneumatic nebulizer

The heated pneumatic nebulizer [36-38] reported by Thomson in 1983 is an API source in which a liquid sample passes through a heated metal tube surrounded by a coaxial nebulizer gas. The combination of heat and gas flow desolvates the nebulized droplets to produce a dry vapor of solvent and analyte molecules which pass through a corona discharge created from a charged needle in the atmospheric pressure region. Heated pneumatic nebulizer ionization can operate with flow rates up to 2 ml/min and it is robust enough to tolerate volatile salts, acids, bases, and other chromatography additives. The technique is a mild ionization source, and it can not offer much structural data from fragmentation.

Liquid ion evaporation

Liquid ion evaporation, developed by Irabarne and Thomson, was reported in 1983 [30]. In this technique, the liquid sample is passed through a pneumatic nebulizer into the atmospheric pressure interface region. Charge is transferred to the droplets from a small high-voltage electrode located near the nebulizer tube. The sample is not heated and conventional flow rates are acceptable. Liquid ion evaporation is well suited for easily ionized and polar analytes, and the mild ionization at room temperature makes liquid ion evaporation very well suited for the production of doubly charged ions [39]. However, the technique can not be used for analytes that are difficult to ionize, and observed ions can experience substantial clustering due to the mild ionization conditions.

Electrospray

In electrospray ionization, the liquid sample is passed through a metal capillary tube at a high-voltage potential, where charge is transferred to the solution before droplets are formed. The technique enables spectra to be obtained from a variety of compounds and offers great flexibility in ion extraction conditions, making possible the formation of fragment, molecular, and cluster ions. The electrospray LC-MS interface will be discussed in greater detail in the next section.

Electrospray - Mass Spectrometry Historical Perspective

The first report of an electrospray-type phenomena dates back almost three hundred years ago to the experiments of Bose [40], while the first description of an actual electrospray was published by Zeleny [41] in 1917. Electrospray (ES) was not used as an ionization source until the late 1960's when Dole and co-workers [42,43] observed ion retardation and ion mobility using ES to produce gas phase macro-ions. Fenn [44] and Aleksandrov [45,46] combined electrospray ionization and mass spectrometry (ES-MS), working independently of each other in 1984. Whitehouse, Fenn, and co-workers [47] gave the first detailed description of an ES interface for LC-MS in 1985.

Although ES-MS offers a relatively simple method for ionizing LC effluent, flow-rate restrictions diminished the usefulness of LC-ES-MS. Ionspray, or pneumatically assisted ES interface, which combines ESI and pneumatic nebulization, was introduced in 1987 [48]. The ionspray interface can introduce flow-rates of 200 μl/min. Increasingly higher flow-rates up to 2ml/min can be achieved currently with minor modifications, such as liquid shield [49] and TurboIonSpray [50].

Electrospray Ionization Process

Although ES-MS is relatively new, the ESI process has been investigated extensively [51-58]. ESI requires four steps: the production of highly charged droplets, solvent evaporation, Rayleigh fission, and finally the production of gas phase ions.

Charged droplet formation

Charged droplets are generally produced by applying a high electric potential to a stainless steel capillary tube, or electrode tube. As the sample solution passes through the electrode tube, charge is transferred to the liquid. A potential difference of 3-6 kV is typically applied between the electrode tube and a counter electrode located about 1 cm away. In pure ESI, the electric stress caused by the potential difference is the sole method of droplet formation. In the more common pneumatically assisted ESI, a nebulizer gas is forced around the electrode tube to facilitate droplet formation. Liquid flow-rate and solvent composition are important factors in droplet production. Pure ESI has typical flow-rates of 1-10 μl/min, while pneumatically assisted ESI has flow rates up to 200 μl/min [48]. Organic solvents, such as methanol or propanol, are required for droplet formation and efficient evaporation, and supporting electrolyte is typically used to assure effective charge transfer to solution droplets.

Solvent evaporation and Rayleigh fission

Droplets formed at the electrode tube experience significant physical changes before reaching the mass spectrometer. In the Perkin Elmer-Sciex API/1 instrument used for this work, the charged droplets are sampled into an interface region, containing a drying, or curtain gas heated to 60°C and bordered by two charged plates as shown in figure 1. The curtain gas stimulates collision-induced dissociation of solvent clusters, and it also prevents clogging of the small, 100 μm diameter, sampling orifice opening. Organic solvents in the solution also increase the evaporation rate and reduce clogging. As the solvent of the charged droplets evaporates, the droplets shrink, and as the droplet shrinks the similar charges inside the droplet are forced closer together. Eventually, the coulombic repulsion between the similar charged ions exceeds the surface tension of the droplet. At this point, the droplet undergoes Rayleigh fission, a process in which small, highly-charged droplets are expelled from the larger initial droplets. These small expelled droplets have been measured to contain 15% of the original droplet's excess charge and only 2% of its original mass. Evaporation and Rayleigh fission can occur repeatedly, producing smaller and smaller droplets. A typical beginning droplet ($r = 1.5$ μm, charge $= 8 \times 10^{-15}$C at 35°C) will go through an average of three fissions in roughly 500 μs before emitting gas phase ions [59].

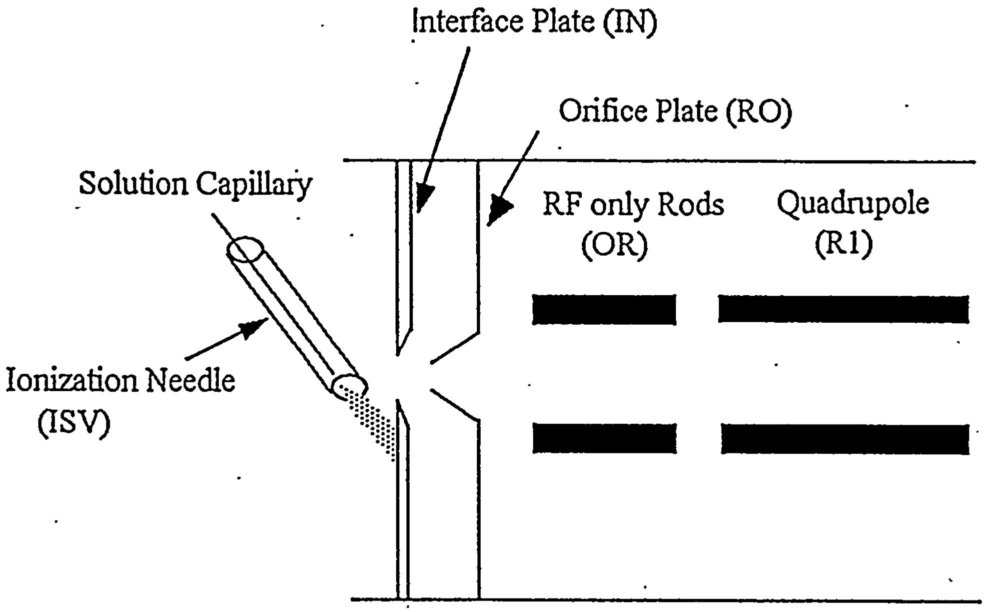

Figure 1: PE-Sciex API/1 Electrospray Mass Spectrometer

Production of gas phase ions

The mechanism that produces gas phase ions from charged droplets is uncertain [60-62]. Two theories have been suggested, ion evaporation theory (IET) [63,64] and single ion droplet theory (SIDT) [42,43,65,66]. The IET was proposed by Iribarne and Thomson in 1976 [63]. It states that a single solvated ion is emitted from the surface of the droplet when the surface charge density of the droplet is strong enough to desorb ions. The SIDT was originally proposed by Röllgen in 1983 [65], and it states that through evaporation and Rayleigh fission the droplets will become so small that they contain only one ion. As this micro-droplet undergoes evaporation, the droplet will be converted to an ion in the gas phase. Experiments to determine the exact mechanism for gas phase ion production have been inconclusive. IET depends on significant charge density on the droplet surface, and SIDT depends on the production of extremely small droplets. Both conditions are feasible in the ESI process.

The gas phase ions produced in the interface region enter a high vacuum region containing the quadrupole mass analyzer through the 100 μm diameter sampling orifice. Most other API source designs are derived from the interface described by Whitehouse et al [47], using a two stage vacuum system, and a sample transfer capillary. The Perkin Elmer - Sciex instrument used for this work incorporates a single quadrupole mass analyzer and a channel electron multiplier for detection.

Dissertation Objectives and Organization

The emphasis of this thesis is the use of LC-ES-MS to identify intermediate and product compounds resulting from the electrochemical incineration (ECI) of organic molecules in aqueous solutions. ECI is a waste remediation process whereby oxygen atoms are transferred from water in the solvent phase to the oxidation products by direct or indirect reactions on the anode surface. ECI is a versatile, energy efficient, environmentally compatible, and low cost method for elimination of organic wastes. The LC-ES-MS techniques described for the identification of ECI unknowns require no sample preparation or analyte derivatization. Limited sample preparation reduces the possibility of altering the analytes, and makes it possible to analyze the sample as it exists in solution.

Chapters 2, 3, 4, and 5 of this thesis stand alone as scientific manuscripts and are either published, submitted for publication or ready for submission. Chapter 6 is a general conclusion with suggestions for future research.

Chapter 2 presents preliminary studies using ion exclusion chromatography (IEC) and ES-MS to analyzes mixtures of carboxylic acids. Compromise experimental parameters were evaluated to observe several organic acids. The effects of sheath gas, solvent composition, concentrated matrix ions, and pH of solution on sensitivity and linearity were examined. A basic isopropanol solvent with nitrogen sheath gas yielded the best results and detection limits from 40 to 200 ppb for direct infusion and 2-8 ppm for IEC-ES-MS. The presence of concentrated matrix ions can suppress the analyte signal significantly. These matrix effects are minimized by chromatographic separation; the use of internal standards compensates for most of the remaining matrix effects. In most cases the sample is injected directly onto the column with little or no preparation. A small additional flow of organic solvent with pH adjustment is added to the sample after separation and before reaching the ES-MS.

Chapter 3 examines the ECI products and intermediates of benzoquinone using the ion exclusion chromatography - ES-MS set-up described in chapter 2. Unknown intermediates and products were identified and studied as a function of ECI time. The ECI procedure is described and electrode efficiency is evaluated. Mechanisms are proposed for the production of maleic, succinic, malonic, and acetic acids.

Chapter 4 presents the study of intermediates and products during the ECI of chlorophenol. In this study a TurboIonSpray ionization source is used with a mass spectrometer. The majority of the unknown determination is accomplished using ion exchange chromatography coupled to the TurboIonSpray mass spectrometer. A suppressor column is also placed between the ion chromatography column and the electrospray interface to eliminate sodium cations from the eluent. Reversed phase chromatography was also used with ES-MS for the determination of unknown ECI samples. The major intermediates and products of ECI were maleic, succinic, malonic, and acetic acids, as well as chloride, chlorate, and perchlorate ions.

Chapter 5 details a make-up liquid sheath flow system for coupling capillary electrophoresis with electrospray mass spectrometry for the determination of carboxylic acids. Maleic, succinic, malonic, and glutaric acids were separated using capillary electrophoresis with an aqueous mobile phase containing 1,2,4,5-benzenetetracarboxylic acid, 2,6-naphthalenedisulfonic acid, methanol, and diethylenetriamine. Optimum mobile phase composition and separation conditions are reported and the system is evaluated using analytical figures of merit such as resolution and detection limits.

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CHAPTER 6. GENERAL CONCLUSION

The main focus of this dissertation has been the use of liquid chromatography (LC) coupled with electrospray mass spectrometry (ES-MS) for the determination of both inorganic and organic compounds in aqueous solution. LC-ES-MS combines retention and structural data to provide a powerful technique for the identification of unknown compounds in complex solutions. Primary focus has been given to electrochemical incineration (ECI) and the determination of ECI products and intermediates.

Chapter 2 presented the study of small carboxylic acids using ion exclusion chromatography (IEC) and ES-MS. Experimental conditions were examined and compromise conditions were determined, while considering pH, sheath gas, mobile phase composition, and matrix effect.

Chapter 3 implemented the IEC-ES-MS method to examine the complex unknown solution produced during ECI of benzoquinone. The ECI procedure and electrode were explained and evaluated. Intermediates and products were determined and examined with respect to incineration time, and mechanisms were proposed for the production of maleic, succinic, malonic, and acetic acids by the ECI of benzoquinone.

The ECI of 4-chlorophenol was studied in chapter 4. Reverse phase chromatography-ES-MS was used for the determination of aromatic compounds, while ion exclusion chromatography-ES-MS was used to study both organic and inorganic ions. The ion exchange chromatography-ES-MS system utilizes a suppressor between the separation column and the ES-MS to remove sodium ions from the mobile phase. Both

LC-ES-MS techniques offer superior separation quality and detection limits compared to IEC-ES-MS. Other techniques including, chemical oxygen demand, total organic carbon, pH and gas chromatography-mass spectrometry, were also used to study the ECI product of 4-chlorophenol. The major intermediates were determined to be benzoquinone, malonic, succinic, and maleic acids, as well as inorganic chlorine-containing anions, such as chloride, chlorate, and perchlorate. Pathways were also suggested for the ECI reaction.

Chapter 5 reported preliminary studies from coupling capillary electrophoresis (CE) with ES-MS. A sheath flow of supporting electrolyte was required for sufficient electrical connection and liquid volume. The mobile phase composition was studied to provide the best compromise results for the observation of four carboxylic acids. Although CE-ES-MS has great potential, with low sample volume and efficient separation, detection limits and reproducibility were poor.

The LC-ES-MS methods used in this work have proven effective for the identification of unknown compounds in complex aqueous solutions. Future work to improve LC-ES-MS may focus on reducing the high background noise present in the ES-MS signal. The addition of TurboIonSpray reduces some noise, but more improvement is needed. One possible area of improvement is the ionization tube and the quality of the spray. The production of small droplets in a well-focused spray can lead to improved ionization efficiency and better sensitivity. Recent work involving nanoelectrospray ionization [1] and ultrasonic nebulization ES-MS [2] seem to be achieving some signal improvements.

The ECI apparatus and procedure successfully oxidizes various organic compounds without producing environmentally hazardous intermediates and products. Most pathways for the formation of CO_2 from larger molecules involve carboxylic acid intermediates. Faster and more efficient ECI may be achieved by focusing on the oxidation of these carboxylic acids. Perhaps the addition of new metal oxides to the existing quaternary metal oxide film will result in faster waste remediation by ECI.

Advancements can also be made with novel sampling methods for ECI studies. One such sampling technique involves pulling ECI products from the electrode surface during ECI to observe the intermediates of the reaction without sampling the bulk of the solution. Sampling at the electrode surface was attempted in this laboratory with mixed results. The rise and fall of intermediate concentrations was observed, as seen for formic acid during the ECI of benzoquinone in figure 1, but the solution sampled represented mostly bulk solution, and provided no specific information about the reactions at the electrode surface. Perhaps a lower liquid flow and more precise and stable inlet placement would reduce the amount of bulk solution sampled and provide more information about the reactions at the electrode surface.

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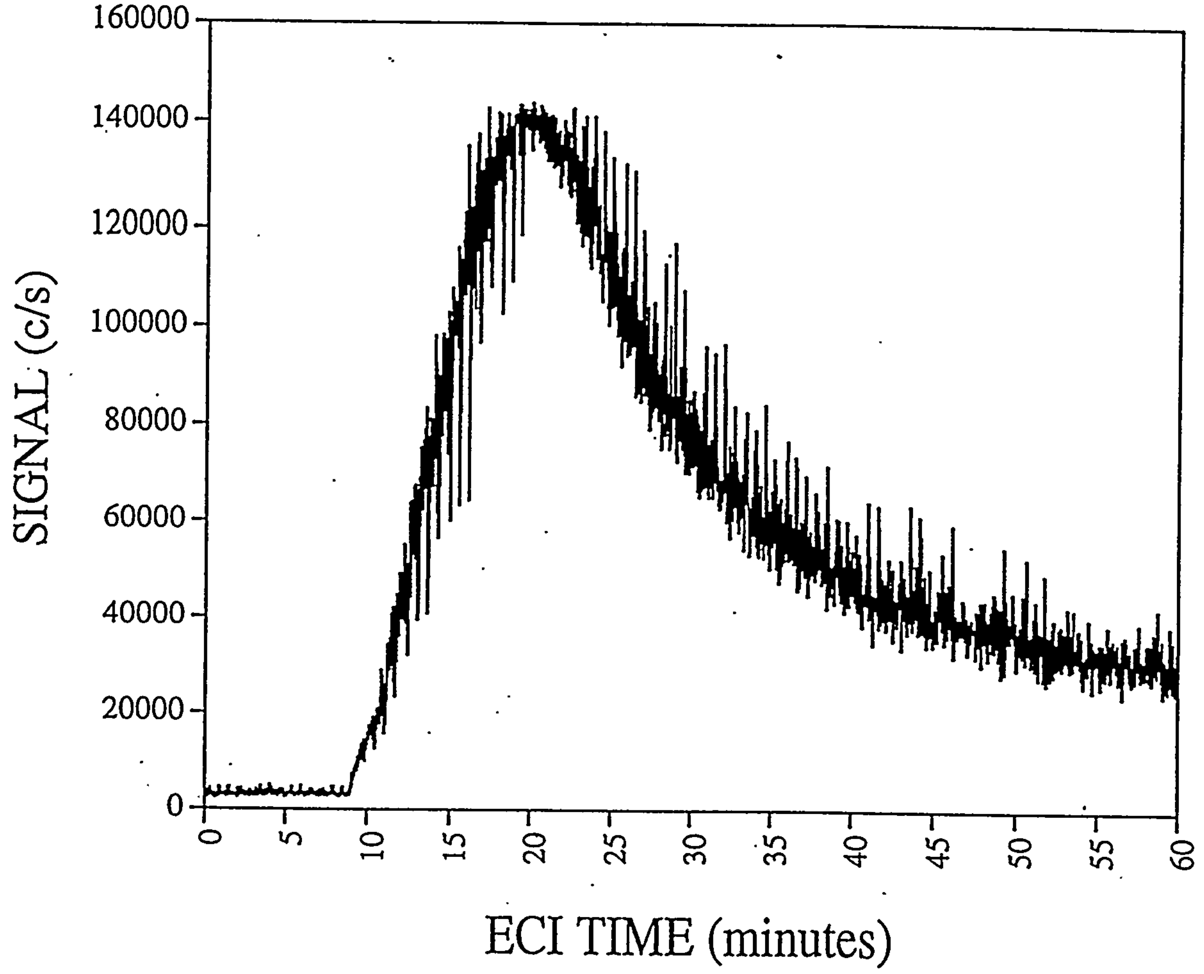

Figure 1. Real-time monitoring of formate ion (m/z=45) near the electrode surface during the ECI of 100 ppm benzoquinone.

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